PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

ANTHONY J. KINNEY
GARY M. FADER

CASE NO.: BB-1071-A

APPLN. NO.: 09/108,010

GROUP ART UNIT: 1638

FILED: JUNE 30, 1998

EXAMINER: E. MCELWAIN

FOR: SUPPRESSION OF SPECIFIC CLASSES OF SOYBEAN SEED PROTEIN GENES

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Anthony J. Kinney Pursuant to 37 CFR §1.132

I am one of the above-identified inventors named in this application. I, Anthony John Kinney, am a citizen of the United Kingdom and am a permanent resident of the United States of America, residing at 609 Lore Avenue, Wilmington, Delaware 19809, and I declare as follows:

I received a B.Sc. in biology from the University of Sussex in 1980 and a D. Phil. in biochemistry and cell biology from Oxford University in 1985. I served as a research fellow in the Department of Food Science at Rutgers University, New Brunswick, N.J. 9/87-5/89. I have been employed at E. I. du Pont de Nemours and Company (DuPont) since June, 1989. I work as a technical leader for DuPont Crop Genetics and am presently working on expression of storage oil, protein and, carbohydrate genes. I have authored in excess of fifteen refereed articles in the field of biochemistry, with emphasis in the field of fatty acid and oil biosynthesis.

2. I have reviewed the Office Action dated March 23, 2001. I am aware that this declaration is being submitted to address the concerns set forth on pages 4 and 5 of the Office Action that "even though the product by process claims are limited and defined by a process, the determination of patentability is based on the product itself."

3. The results shown in the application indicate that the transgene responsible for the phenotype was integrated into a single locus. Example 2 of the application, in particular page 24 at lines 4 through 13, describes the isolation of transgenic soybean

sublines (G94-1, G94-19) with high oleic acid and suppressed β-conglycinin subunits derived from transformation event 260-05. The sublines are described as containing two copies of the plasmid pBS43 at a ***single*** locus, called the "transwitch locus" in example 2 and *locus A* in this declaration. *Locus* A is responsible for both the high oleic and the β-conglycinin null phenotype. Example 2 also states that R5 seeds derived from both of these sublines (G94-1, G94-19) have suppressed β-conglycinin subunits. (For clarity it should be noted that the terms "Transwitch locus" and "*locus A*" are used interchangebly herein.)

4. The Southern blot analyses presented below further analyze the transgenic seeds and plants and show that the phenotype is due to the presence of an insertion at a single locus. The experiments discussed herein were performed by me or others working under my guidance and direction or in coordination with the DuPont regulatory group.

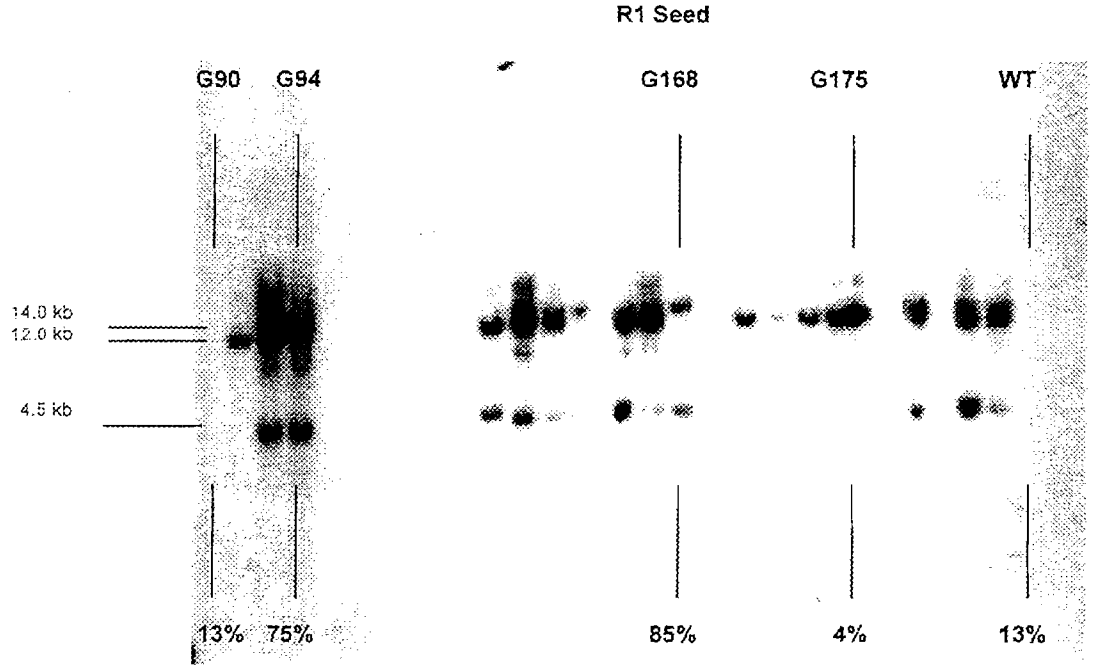
5. The nature of the insert at *locus A* ("transwitch locus") was initially determined by Southern blot analyses of DNA from leaves of R1 and R2 plants. Genomic DNA was digested with Bam HI and probed with the 3' region of phaseolin to detect the *GmFad 2-1* gene expression cassette. Bam HI cuts once in the plasmid and would be expected to result in one hybridising band for each copy of the plasmid inserted into the genome. The results of Southern blot analysis of DNA isolated from leaf tissue of event 260-05 R1 plants that were grown from chipped seeds analysed for fatty acid composition are shown below in Figure 1.

The DNA hybridisation pattern depicted in Figure 1 shows clearly that in the original transformation event the *GmFad 2-1* construct was integrated at two different loci in the soybean genome. At *locus A* the *GmFad 2-1* construct silenced the endogenous *GmFad 2-1* gene, resulting in seeds with an oleic acid content generally above 80%. *Locus A* contained two copies of the *GmFad 2-1* expression cassette as indicated by the two hybridising fragments of 14.0 kb and 4.5 kb. The second locus (*locus B*) contained a copy of *GmFad 2-1* that was over-expressing thus decreasing oleic acid levels to around 4%. *Locus B* contained only one copy of the *GmFad 2-1* expression cassette as noted by the single hybridising fragment of 12.0 kb.

Since G94 contained both loci in the R1 plant an additional round of selection was necessary on the segregating R2 plants to isolate plants containing *locus A* and not *locus B*. Southern blot analysis on genomic DNA isolated from leaf tissue of R2 plants grown from G94 R2 seed using Bam HI digestion and the phaseolin 3' probe identified two sublines, G94-1 and G94-19, that contained *locus A* but not *locus B* since *locus B* had been removed by segregation. *Locus B* was not further

characterized. Figure 2 shows Southern blot analysis of R1 and R2 leaf tissue originating from 260-05 G94 R1 seed. The genomic DNA was digested with Bam HI and probed with the phaseolin 3' probe to detect the integration of the GmFad 2-1 construct.

Figure 1



Additional Southern blot analyses using DNA from the leaves of R6 plants, using multiple restriction enzymes, confirmed that G94-1 and G94-19 contained a single transgenic locus (*locus A*, the "Transwitch locus"). Figure 3 shows the results of Southern blot analyses of DNA isolated from R6 leaf tissue of G94-1 and G94-19, of a sister line from the same event (G168), and of control elite soybean line A2396. In this figure DNA was digested with either Bam HI, Bsp HI, Hind III, or Sst I and hybridized with the phaseolin 3' probe. The Bam HI pattern is identical to the R2 plants.

7. The results mentioned in the application and expanded upon above indicate that suppression of β-conglycinin subunits is caused by an insertion in a single locus of the transgenic plant.

Figure 2

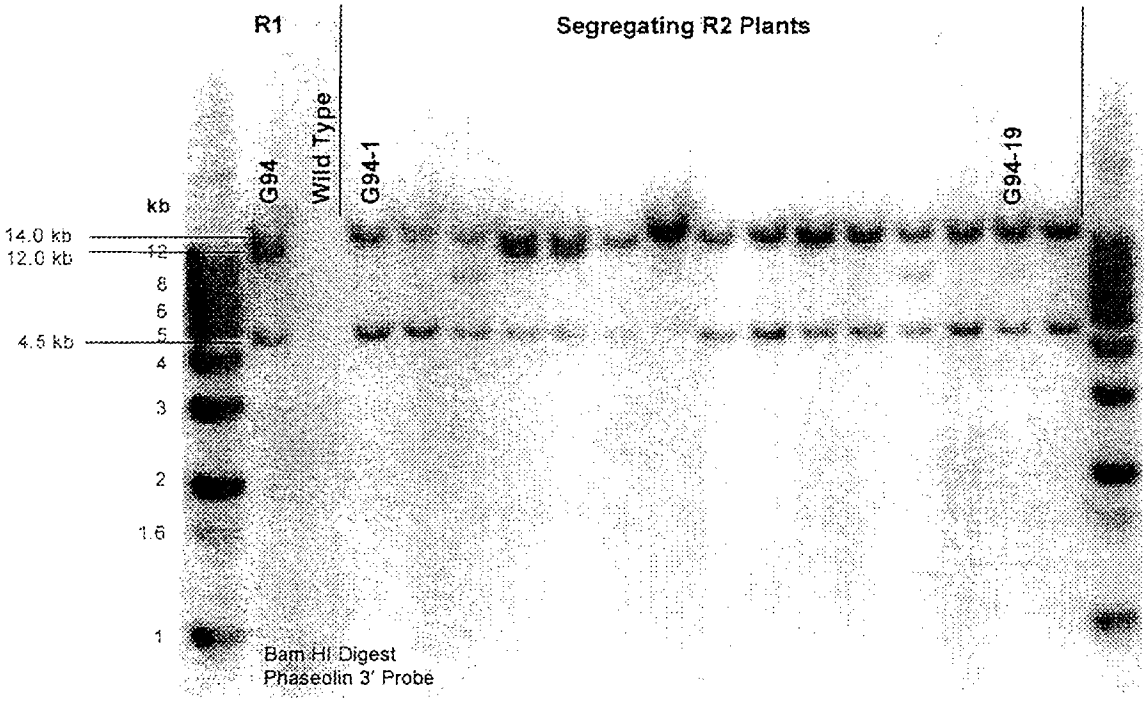
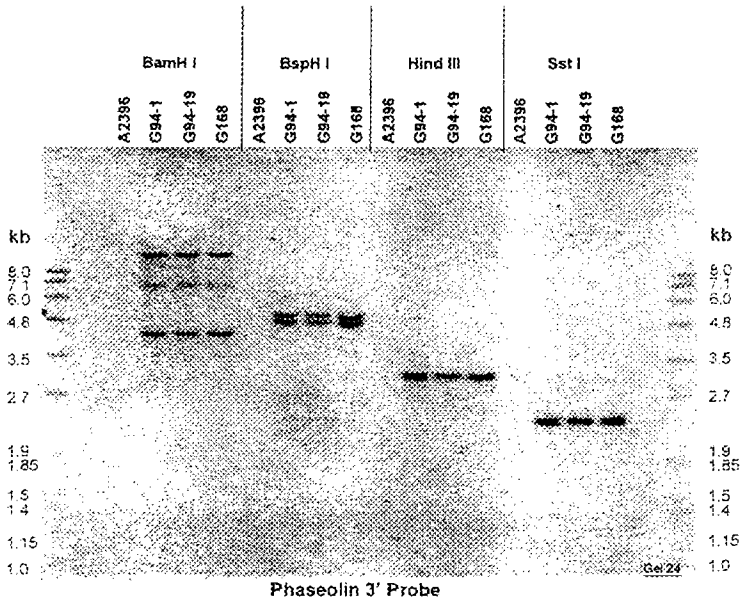
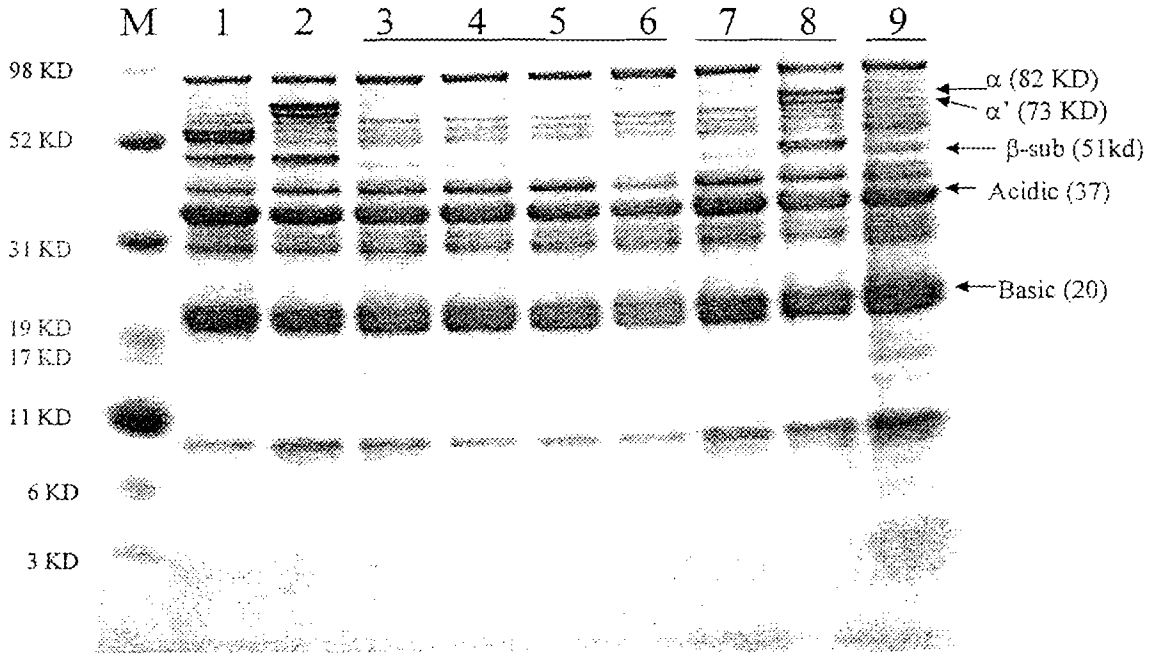


Figure 3



8. Furthermore, analysis, as described in Example 3, of other transgenic soybean lines containing exactly the same α'-subunit promoter sequence of the construct in Example 2 indicates that R1 seeds of these events lack the α, α', and sometimes β subunits of β-conglycinin. Figure 4 shows a picture of a Coomassie brilliant blue R-stained SDS polyacrylamide gel where 20 μg total protein was loaded per lane. The origin of the material loaded on each lane is indicated below the picture of the gel.Figure 4

Figure 4



1. 260-05(G94-1)(85%18:1)
2. JACK (20%)
3. 2308-22-16-H5 (80%)
4. 2308-22-3-14 (80%)
5. 2308-23-5-H1 (72%)
6. 2308-23-6-H13 (72%)
7. 2323-31-1-H34(82%)
8. 2323-31-10-9 (82%)
9. 2335-26-14-11 (80%)
10. 2335-26-2-8 (80%)

In summary, all of the elements of the claimed invention were provided in the patent application. The data presented in this declaration are consistent with the disclosure set forth in the specification.

Accordingly, one skilled in the art can take these elements, as discussed above, and practice the invention without undue experimentation.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Anthony J. Kinney

29 JUNE 2001

Date